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Acclimation of peanut (*Arachis hypogaea* L.) leaf photosynthesis to elevated growth CO_2 and temperature

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Abstract

Peanut (*Arachis hypogaea* L. cv. Florunner) was grown from seed sowing to plant maturity under two daytime CO_2 concentrations ($[CO_2]$) of 360 μmol mol^{-1} (ambient) and 720 μmol mol^{-1} (elevated) and at two temperatures of 1.5 and 6.0 °C above ambient temperature. The objectives were to characterize peanut leaf photosynthesis responses to long-term elevated growth $[CO_2]$ and temperature, and to assess whether elevated $[CO_2]$ regulated peanut leaf photosynthetic capacity, in terms of activity and protein content of ribulose bisphosphate carboxylase-oxygenase (Rubisco), Rubisco photosynthetic efficiency, and carbohydrate metabolism. At both growth temperatures, leaves of plants grown under elevated $[CO_2]$ had higher midday photosynthetic CO_2 exchange rate (CER), lower transpiration and stomatal conductance and higher water-use efficiency, compared to those of plants grown at ambient $[CO_2]$. Both activity and protein content of Rubisco, expressed on a leaf area basis, were reduced at elevated growth $[CO_2]$. Declines in Rubisco under elevated growth $[CO_2]$ were 27–30% for initial activity, 5–12% for total activity, and 9–20% for protein content. Although Rubisco protein content and activity were down-regulated by elevated $[CO_2]$, Rubisco photosynthetic efficiency, the ratio of midday light-saturated CER to Rubisco initial or total activity, of the elevated-$[CO_2]$ plants was 1.3- to 1.9-fold greater than that of the ambient-$[CO_2]$ plants at both growth temperatures. Leaf soluble sugars and starch of plants grown at elevated $[CO_2]$ were 1.3- and 2-fold higher, respectively, than those of plants grown at ambient $[CO_2]$. Under elevated $[CO_2]$, leaf soluble sugars and starch, however, were not affected by high growth temperature. In contrast, high temperature reduced leaf soluble sugars and starch of the ambient-$[CO_2]$ plants. Activity of sucrose-P synthase, but not adenosine 5′-diphosphoglucose pyrophosphorylase, was up-regulated under elevated growth $[CO_2]$. Thus, in the absence of other environmental stresses, peanut leaf photosynthesis would perform well under rising atmospheric $[CO_2]$ and temperature as predicted for this century.

Keywords: *Arachis hypogaea*; CO_2 enrichment; High temperature; Nonstructural carbohydrates; Photosynthesis; Rubisco

1. Introduction

The current atmospheric carbon dioxide concentration ($[CO_2]$) of 360–370 μmol mol^{-1} limits the photosynthetic capability, growth and yield of many

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agricultural crop plants, with C_3 species showing great potential for response to rising [CO_2] (Kimball et al., 1993; Drake et al., 1997). With rapid increases in world industrial development, fossil fuel dependence and changing land use practices, the atmospheric [CO_2] is expected to double within this century. As a result, global climate changes, including alterations in precipitation patterns and significant increases in global air temperatures, possibly as much as 4–6 °C, may occur in the coming decades (Kattenberg et al., 1996; Morison and Lawlor, 1999; Schneider, 2001).

Leaf photosynthetic CO_2 exchange rate (CER) is primarily controlled by ribulose bisphosphate carboxylase-oxygenase (Rubisco), the enzyme responsible for fixing atmospheric CO_2 into the photosynthetic carbon reduction cycle. Rubisco is impacted by atmospheric [CO_2] as well as other environmental factors, including air temperature, sunlight and soil moisture. In C_3 plants, Rubisco activity is CO_2-limited under present atmospheric conditions, and increased [CO_2] enhances CER. However, in a number of C_3 plants, long-term exposure to elevated growth [CO_2] is often followed by physiological changes, resulting in decreased leaf photosynthetic capacity which is manifested through reductions in activity and protein concentration of Rubisco (Vu et al., 1997; Gesch et al., 1998; Moore et al., 1998, 1999). However, the down-regulation of leaf photosynthetic capacity at elevated growth [CO_2] is not a universal phenomenon, as there are species showing no changes in Rubisco under this condition (Bowes, 1993).

Long-term experiments on climate change under controlled environmental conditions have focused primarily on temperate plant species (Sage et al., 1989; Van Oosten and Besford, 1995; Nie et al., 1995; Moore et al., 1998; Gesch et al., 1998; Vu et al., 1997, 1999, 2001). Few studies have examined the effects of increased [CO_2] and its interaction with other climate change factors on the physiology of subtropical and tropical crops. Peanut or groundnut (*Arachis hypogaea* L.), a subtropical pulse or oil crop, has been widely grown under various climatic conditions in Africa, Asia, and North and South America. However, assessment of the regulatory mechanisms of peanut photosynthesis in response to future changes in climatic conditions is limited (Clifford et al., 2000).

In this study, peanut was grown from seed sowing to plant maturity under ambient and double-ambient [CO_2] and at near outdoor ambient and 6.0 °C above outdoor ambient temperatures. The hypothesis being tested here was that, in peanut, leaf photosynthetic capacity was down-regulated and leaf Rubisco photosynthetic efficiency and carbohydrate metabolism were up-regulated under long-term elevated growth [CO_2]. In addition, peanut leaf photosynthesis response to both elevated growth [CO_2] and temperature will be assessed.

2. Materials and methods

2.1. Plant material and growth conditions

Uniform seeds of peanut (*A. hypogaea* L. cv. Florunner) were selected and inoculated prior to sowing with peanut-type *Bradyrhizobium* (Nitragin, Liphatech, Milwauki, WI). They were planted on 9 July 1999 and grown in Arredondo fine sand (loamy, siliceous, hyperthermic Grossarenic Paleudult) in two temperature-gradient greenhouses (TGGs) controlled at 1.5 and 6 °C above outdoor ambient temperature. The [CO_2] was maintained at ambient (360 μmol mol^{-1}) in one TGG, and 360 μmol mol^{-1} above ambient (elevated, 720 μmol mol^{-1}) in the other. Temperature and CO_2 controls were based on the TGG infrastructure used by Okada et al. (1995) and the modified hardware as described by Sinclair et al. (1995) and Fritschi et al. (1999). Briefly, these TGGs, with semi-cylindrical galvanized steel arch structures, were 27.4 m long, 4.3 m wide, and 2.2 m high at the ridgepole. A computer-controlled, variable-speed ventilation fan mounted at the south end of each TGG controlled airflow and regulated the temperature gradient, which averaged from 1.5 °C above outside ambient temperature (T_A) at the air-entry north end (segment 1) to 6.0 °C above T_A at the south end (segment 4). During much of the time, heat was provided by two 1500 W electric heaters mounted on each side along the length of the TGG at 5.5 m increments. Incoming solar radiation supplanted the need for continuous electrical heat during bright weather, and the heaters were turned off and on in concert with the variable speed fan to provide an average temperature gradient of 4.5 °C between segments 1 and 4. The ventilation fan speed and electric heaters were controlled by microprocessor algorithm. Over-

head paddle fans at the beginning of each segment mixed the heated air to minimize vertical gradients of temperature. Carbon dioxide enrichment was implemented during daylight hours by injection of CO_2 at 1.8 m into the air-entry segment of the TGG through a predilution system that provided cross-sectional uniform CO_2 concentrations. Temperature and [CO_2] were controlled and monitored for each TGG individually. Dewpoint temperatures were measured in the outside ambient air and at the warmest end of the TGGs. A Keithley-Metrabyte supervised controller/data acquisition system (SCADA) (Woburn, MA) with Intellution FIX DMACS supervisory software (Intellution, Inc., Norwood, MA) was used to measure temperatures, calculate temperature gradients, adjust ventilation rates, control heaters, measure CO_2 concentration, control CO_2 injection rates, and log data. The quality of CO_2 and temperature controls for these TGGs was given by Newman et al. (2001).

Irrigation was applied three times per week with a double-overlapping microjet sprinkler system, providing 7–8 mm water per day during the plant growth period, as described by Newman et al. (2001). As the soil was rich in phosphorous and potassium, plants did not receive any inorganic or organic fertilizer, except for gypsum dust application (75 g m^{-2}) at flowering due to high calcium requirement of peanut (Prasad et al., 2003). Nitrogen nutrition was dependent on symbiotic dinitrogen fixation. The plot size for each CO_2-temperature treatment inside the two TGGs used for the experiment was 2 m × 5 m, and the plant density was 30 plants m^{-2} at the time of leaf gas exchange measurements and leaf sampling for enzyme and carbohydrate analyses.

2.2. Leaf gas exchange measurements

Photosynthetic CO_2 exchange rate, stomatal conductance and transpiration of single, attached, uppermost fully expanded mature leaflets were measured during 10–24 September 1999 at midday, between 1100 and 1400 eastern day time (EDT) when solar photosynthetic photon flux density (PPFD) was at 1400–2200 μmol m^{-2} s^{-1}, using the LI-6200 Portable Photosynthesis System (LI-COR, Inc., Lincoln, NE) and a 1 dm^3 leaf chamber. Gas exchange measurements were determined at the [CO_2] and temperature used for growth. For each of the four CO_2-temperature treatments, i.e., ambient $CO_2/T_A + 1.5\,^{\circ}C$, ambient $CO_2/T_A + 4.5\,^{\circ}C$, double-ambient $CO_2/T_A + 1.5\,^{\circ}C$, and double-ambient $CO_2/T_A + 4.5\,^{\circ}C$, measurements were made on 17–19 leaflets randomly selected from 17 to 19 plants. For each measurement, right before enclosing the leaflet in the chamber, [CO_2] and temperature in the air running through the LI-COR system, with the leaf chamber left opened, were monitored. After an equilibrium with the levels of CO_2 and temperature used for growth was reached, the leaflet was quickly placed in the leaf chamber, which was then closed and latched, and the system computer was directed to begin making measurement. As the duration for each measurement was typically 15–20 s, leaf temperatures throughout the midday measurements were very close to those of chamber air temperatures. During the 2-week measurement period, there were variations in solar irradiance and ambient air temperature within and among days. On 10 September, a clear day, ambient air temperature was 33.9 °C at 1104 EDT (1880 μmol m^{-2} s^{-1} PPFD) and 35.6 °C at 1305 EDT (2200 μmol m^{-2} s^{-1}). On 24 September, also a clear day, ambient air temperature was 34.2 °C at 1100 EDT (2050 μmol m^{-2} s^{-1} PPFD) and 36.4 °C at 1245 EDT (2100 μmol m^{-2} s^{-1} PPFD). During the midday measurements on 13 September, an overcast day, solar PPFD fluctuated between 800 and 2400 μmol m^{-2} s^{-1}, while ambient air temperature varied from 33.5 to 37.4 °C. Therefore, only data obtained at solar PPFD of 1400–2200 μmol m^{-2} s^{-1} were selected.

In addition, a measurement of leaf CER responses to declines in CO_2 concentration of the air inside the closed chamber (C_{ca}) containing the attached leaf, i.e., CER versus C_{ca} response curve, was constructed through the "drawdown" procedure using the closed LI-6200 System as described by McDermitt et al. (1989), as leaf assimilation causes C_{ca} of the chamber air to begin to drop as soon as the leaf is enclosed in the chamber. The CER versus C_{ca} curve was performed in situ on uppermost mature leaflets for both ambient and double-ambient [CO_2] peanut plants of the $T_A + 1.5\,^{\circ}C$ treatment. Measurements were done between 1000 and 1100 EDT (1400–1500 μmol m^{-2} s^{-1} PPFD) on 16 September by enclosing the single attached leaflet in the 1 dm^3 assimilation chamber of the LI-6200 and allowing the leaflet to remove CO_2. After equilibrating for ∼30 s, leaflet CER was monitored

over CO_2 changes, as C_{ca} inside the closed chamber gradually dropped toward the compensation point.

2.3. Leaf sampling and analyses of enzymes and carbohydrates

Uppermost fully expanded leaflets were sampled at midday from 1230 to 1300 EDT on 24 September 1999, a clear day with solar PPFD of $\sim$1800 μmol m^{-2} s^{-1}. At each sampling, 36 leaflets were detached from nine different plants for each treatment and immediately immersed in liquid N_2. Sampled leaflets were pooled by treatment, ground to a fine powder in liquid N_2 with a mortar and pestle, and stored in liquid N_2 until analyses. Subsequently, triplicate subsamples of the liquid N_2-frozen leaf powder were analyzed for activities of Rubisco, sucrose-P synthase (SPS) and adenosine 5′-diphosphoglucose pyrophosphorylase (ADGP) and concentration of Rubisco protein, as previously reported (Vu et al., 2001).

Additionally, subsets of 36 leaflets were also sampled at the same time from nine different plants for each treatment for determinations of leaf fresh weight and area. Leaf samples were then oven-dried at 60 °C, and leaf dry weights were measured. Oven-dried leaflets were pooled by treatment and ground to a powder, and triplicate subsamples of the oven-dried leaf powder were used for carbohydrate measurements. Soluble sugars were extracted from approximately 100 mg of the oven-dried leaf powder with 80% (v/v) ethanol at 85 °C. Glucose, fructose and sucrose were quantified using the microtiter method (Hendrix, 1993). Pellets containing starch were oven-dried overnight at 60 °C. Starch in the pellet was first gelatinized by addition of 1 ml of 0.2N KOH and incubation in a boiling water bath for 30 min (Rufty and Huber, 1983). After cooling, 0.2 ml of 1 M acetic acid was added, and the solution was incubated with 2 ml acetate buffer (pH 4.6) containing amyloglucosidase (6 units, Boehringer Mannheim) at 55 °C for 1 h. The reaction was terminated in a boiling water bath, and the resulting supernatant was analyzed for glucose.

2.4. Statistical analyses

The experimental design was that of a split-plot, with CO_2 as the main plot, and temperature as the subplot treatments. As there was only one TGG per CO_2 concentration, the difference between CO_2 concentrations was confounded with the difference between the TGGs. Values presented for leaf gas exchanges are the means of determinations on 17–19 leaflets (one leaflet per plant). Values presented for the enzymes and carbohydrates are the means of triplicate determinations from each of three subsamples from the combined pool of 36 leaflets harvested from nine different plants, and those presented for leaf biomass and area are the means of 36 leaflets harvested from nine different plants. Differences among treatment means were determined using the Duncan Multiple Range Test.

3. Results

Peanut plants grown and measured at 720 μmol CO_2 mol^{-1} had higher leaf CER than their counterparts at 360 μmol [CO_2] mol^{-1} at both growth temperatures (Table 1). Percent enhancement in CER by

Table 1
Midday CO_2 exchange rate (CER), transpiration, stomatal conductance and water-use efficiency (WUE) of fully-developed leaves of 'Florunner' peanut plants grown for a season under 360 and 720 μmol CO_2 mol^{-1} and at average temperatures of 1.5 and 6.0 °C above outdoor ambient temperature (T_A)

[CO_2] (μmol mol^{-1})	Temperature (°C)	CER (μmol CO_2 m^{-2} s^{-1})	Transpiration (mmol H_2O m^{-2} s^{-1})	Conductance (mmol H_2O m^{-2} s^{-1})	WUE (mmol CO_2 mol^{-1} H_2O)
360	T_A + 1.5	32.4 (1.0) b	7.2 (0.3) ab	522 (27) b	4.5 (0.2) b
	T_A + 6.0	34.2 (1.1) b	7.8 (0.4) a	652 (30) a	4.4 (0.3) b
720	T_A + 1.5	43.8 (1.7) a	6.3 (0.4) c	366 (32) c	7.0 (0.4) a
	T_A + 6.0	39.9 (1.5) a	6.5 (0.4) bc	363 (39) c	6.2 (0.3) a

Measurements were performed 62–76 days after seed planting. Values are the mean and S.E. (parentheses) of 17–19 determinations. Values with different letters in the same column are significantly different at $P < 0.05$ using a Duncan Multiple Range Test.

elevated [CO_2] was 33% under near-ambient growth temperature ($T_A + 1.5\,^{\circ}C$), and 17% under high growth temperature ($T_A + 6\,^{\circ}C$). In contrast, leaf transpiration and stomatal conductance rates were lower under elevated [CO_2]. Leaf transpiration of the elevated-CO_2 plants, compared with those of the ambient-CO_2 controls, was 12% less at near-ambient temperature and 17% less at high temperature. Similarly, leaf stomatal conductance of the elevated-CO_2 plants was 30 and 44% lower than that of their ambient-CO_2 counterparts at near-ambient and high temperature, respectively. There was also a small, although not significant, increase in leaf transpiration for both CO_2 treatments under high temperature (Table 1). Furthermore, high temperature increased leaf stomatal conductance by 25% for the ambient [CO_2] treatment, but hardly affected that of the elevated [CO_2] treatment.

Leaf photosynthetic water-use efficiency (WUE), the ratio of leaf CER to leaf transpiration rate, was higher for plants grown at elevated [CO_2] than those at ambient [CO_2] (Table 1). Under near-ambient temperature, WUE of the elevated [CO_2]-grown plants was 56% greater at near-ambient temperature and 41% greater at high temperature. High temperature per se slightly reduced leaf WUE (2–13% less).

A typical pair of response curves of leaf CER versus CO_2 concentration of the air inside the closed assimilation leaf chamber (C_{ca}) is shown in Fig. 1 for both ambient and double-ambient [CO_2] peanut plants of the $T_A + 1.5\,^{\circ}C$ treatment. Leaf CER continued to drop as C_{ca} declined. Extrapolation of each CER response curve gave an interception at the abscissa of about 95 μmol CO_2 mol^{-1}, which represented the CO_2 compensation point for peanut leaves of both CO_2 treatments. The initial slope of the CER response curve for plants grown at elevated [CO_2], however, was about 0.073, compared with 0.123 for that of plants grown at ambient [CO_2] (Fig. 1). A similar pair of response curves obtained at another day (not shown) also showed different initial slopes for the two CO_2 treatments.

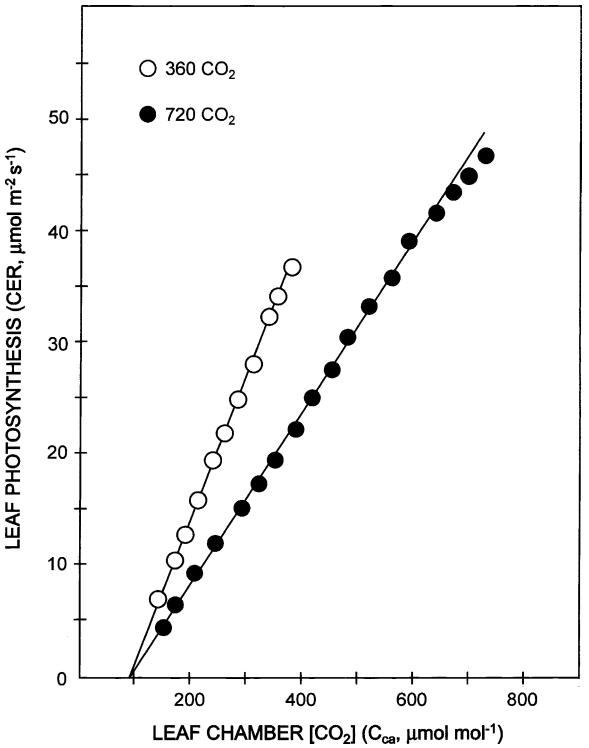

Fig. 1. Leaf CER response curves for the 360 μmol CO_2 mol^{-1} (ambient) and 720 μmol CO_2 mol^{-1} (elevated) peanut plants of the $T_A + 1.5\,^{\circ}C$ treatment. Measurements were performed in situ on uppermost mature leaves, 68 days after seed planting, from 1000 to 1100 EDT (1400–1500 μmol m^{-2} s^{-1} solar PPFD). The single attached leaflet was enclosed in the 1 dm^3 assimilation chamber of the LI-6200 Portable Photosynthesis System, and CER responses to declines in CO_2 concentration of the air inside the closed chamber (C_{ca}) were monitored through the "drawdown" procedure described by McDermitt et al. (1989). Extrapolation of the curves gave an interception at the abscissa of about 95 μmol CO_2 mol^{-1} for both CO_2 treatments. The initial slopes of the CER response curves were 0.123 for the ambient-CO_2 plants and 0.073 for the elevated-CO_2 plants.

Growth at elevated [CO_2] resulted in a down-regulation of both activity and protein content of Rubisco, expressed on a leaf area basis (Table 2). Elevated [CO_2] reduced the initial activity of Rubisco by 27% at near-ambient temperature and 30% at high temperature. Reductions in total activity by elevated [CO_2], however, were less: about 5% at near-ambient temperature and 12% at high temperature. Similarly, reductions in Rubisco protein content by long-term elevated growth [CO_2] were about 15% at near-ambient temperature and 20% at high temperature. In addition, Rubisco activation, the ratio of the initial to the corresponding total activity, was also reduced under CO_2 enrichment. Rubisco activation was 73 and 74% under ambient growth [CO_2], compared to 57 and 58% under elevated growth [CO_2], for the near-ambient and elevated temperature treatments, respectively.

Table 2
Activity, activation, protein content and photosynthetic efficiency of Rubisco in midday-sampled, fully-developed leaves of 'Florunner' peanut plants grown for a season under 360 and 720 μmol CO_2 mol^{-1} and at average temperatures of 1.5 and 6.0 °C above outdoor ambient temperature (T_A)

$[CO_2]$ ($\mu mol\ mol^{-1}$)	Temperature (°C)	Activity		Activation (%)	Protein content ($g\ m^{-2}$ leaf area)	Photosynthetic efficiency	
		Initial ($\mu mol\ m^{-2}$ leaf area s^{-1})	Total ($\mu mol\ m^{-2}$ leaf area s^{-1})			CER/$R_{initial}$ (%)	CER/R_{total}
360	T_A + 1.5	38.0 (1.5) a	51.3 (2.4) ab	74.1	2.25 (0.12) a	85.3	63.2
	T_A + 6.0	41.1 (1.8) a	56.3 (2.3) a	73.0	2.23 (0.11) a	83.2	60.8
720	T_A + 1.5	27.6 (2.0) b	48.5 (1.8) b	56.9	1.92 (0.10) b	158.7	90.3
	T_A + 6.0	28.7 (2.2) b	49.4 (2.4) b	58.1	1.78 (0.12) b	139.0	80.8

Leaf sampling was performed at midday (~1800 $\mu mol\ m^{-2}\ s^{-1}$ PPFD), 76 days after seed planting. Values are the mean and S.E. (parentheses) of three determinations. Rubisco activation is computed as the ratio of initial to total activity. Rubisco photosynthetic efficiency is computed as the ratio of midday leaf CER (Table 1) to Rubisco initial ($R_{initial}$) and total (R_{total}) activity. Values with different letters in the same column are significantly different at $P < 0.05$ using a Duncan Multiple Range Test.

Despite a down-regulation of both Rubisco activity and protein concentration, Rubisco photosynthetic efficiency, the ratio of midday light-saturated CER to Rubisco activity, was higher for plants grown under elevated $[CO_2]$ (Table 2). Rubisco photosynthetic efficiency was 1.9- and 1.7-fold higher when computed in terms of initial Rubisco activity, and 1.4- and 1.3-fold greater when computed in terms of total Rubisco activity, for the elevated $[CO_2]$ plants grown at near ambient temperature and high temperature, respectively, compared with those of the ambient $[CO_2]$ plants at corresponding growth temperatures.

Nonstructural carbohydrates, expressed on a leaf area basis, were higher in midday-sampled leaves of plants grown at elevated $[CO_2]$ than those grown at ambient $[CO_2]$ regardless of growth temperature (Figs. 2 and 3). Under elevated $[CO_2]$, reducing sugars (glucose + fructose) increased by 31 and 39%, sucrose by 20 and 99% and total soluble sugars by 26 and 59% at near-ambient and high growth temperature, respectively (Fig. 2). Similarly, starch increased by 93 and 100% and total nonstructural carbohydrates by 82 and 94% for the elevated-CO_2 plants at near-ambient and high temperature, respectively (Fig. 3). At ambient $[CO_2]$, high growth temperature reduced the levels of reducing sugars by 16%, sucrose by 29% and total soluble sugars by 21% (Fig. 2), and starch by 6% and total nonstructural carbohydrates by 9% (Fig. 3). However, at elevated $[CO_2]$, the reductions in carbohydrates by high temperature were less: 11% for reducing sugars and only about 3% for both starch and total nonstructural carbohydrates. Total soluble sugars were not affected and sucrose was even slightly increased (~18% more) for the elevated-CO_2-grown plants under high temperature.

Table 3 shows activities of SPS and ADGP in midday-sampled leaves of peanut grown under two $[CO_2]$ and temperature treatments. In terms of growth $[CO_2]$, activities of SPS were up-regulated under elevated $[CO_2]$ at near ambient temperature (~29% higher), but not at high temperature. There was about 10% decline in SPS activity in elevated CO_2-grown

Table 3
Activities of sucrose-P synthase (SPS) and adenosine 5′-diphosphoglucose pyrophosphorylase (ADGP) in midday-sampled, fully-developed leaves of 'Florunner' peanut plants grown for a season under 360 and 720 μmol CO_2 mol^{-1} and at average temperatures of 1.5 and 6.0 °C above outdoor ambient temperature (T_A)

$[CO_2]$ ($\mu mol\ mol^{-1}$)	Temperature (°C)	Enzyme activity	
		SPS ($\mu mol\ m^{-2}$ leaf area s^{-1})	ADGP
360	T_A + 1.5	4.1 (0.3) c	25.8 (1.9) a
	T_A + 6.0	4.5 (0.3) bc	25.9 (0.8) a
720	T_A + 1.5	5.3 (0.2) a	27.4 (0.4) a
	T_A + 6.0	4.8 (0.3) ab	27.3 (0.8) a

Leaf sampling was performed 76 days after seed planting. Values are the mean and S.E. (parentheses) of three determinations. Values with different letters in the same column are significantly different at $P < 0.05$ using a Duncan Multiple Range Test.

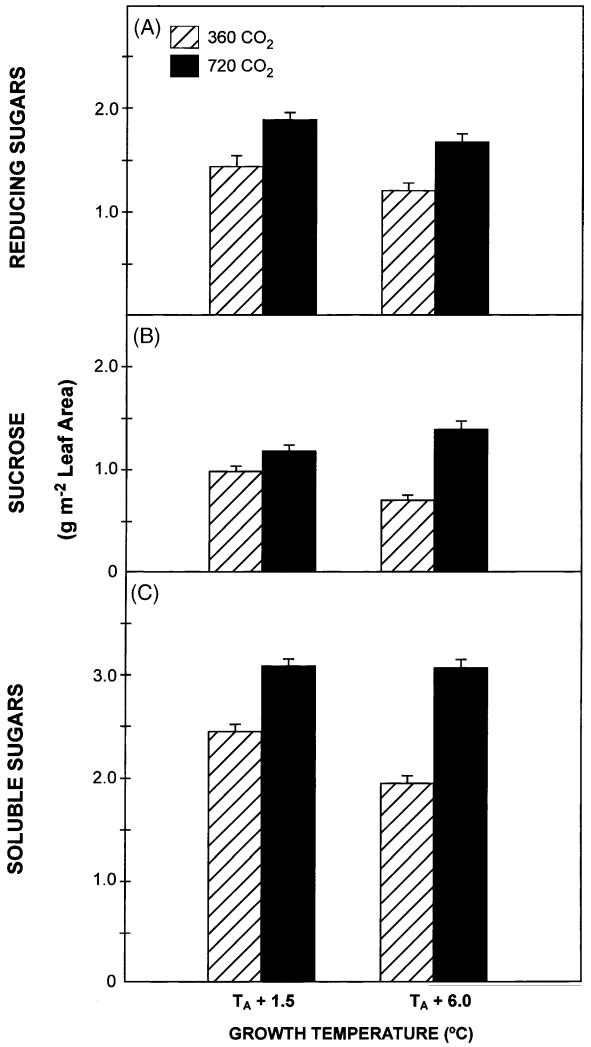

Fig. 2. Concentrations of reducing sugars (A), sucrose (B) and soluble sugars (reducing sugars + sucrose) (C) in leaves of peanut plants grown for a season at 360 and 720 μmol CO_2 mol^{-1} and under near ambient ($T_A + 1.5\,°C$) and high ($T_A + 6.0\,°C$) temperature. Uppermost fully expanded leaflets were sampled at midday (~1800 μmol m^{-2} s^{-1} solar PPFD), 76 days after seed planting. Values are the mean of three determinations. Bars represent S.E.

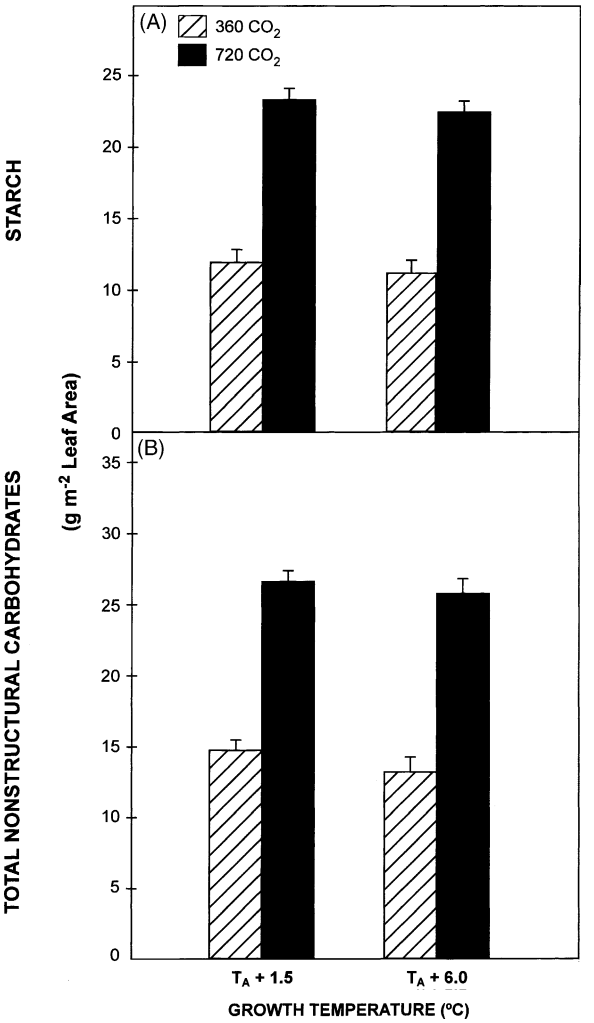

Fig. 3. Concentrations of starch (A) and total nonstructural carbohydrates (starch + soluble sugars) (B) in leaves of peanut plants grown for a season at 360 and 720 μmol CO_2 mol^{-1} and under near ambient ($T_A + 1.5\,°C$) and high ($T_A + 6.0\,°C$) temperature. Uppermost fully expanded leaflets were sampled at midday (~1800 μmol m^{-2} s^{-1} solar PPFD), 76 days after seed planting. Values are the mean of three determinations. Bars represent S.E.

plants exposed to high temperature. Activity of the enzyme of ambient CO_2-grown plants, however, was about 10% greater at high temperature. Although not significantly different, activities of ADGP were consistently 5–6% greater under elevated growth [CO_2]. Activities of ADGP, however, were not affected by high growth temperature.

Leaf biomass, area and specific weight were enhanced by elevated growth [CO_2] (Table 4). At near-ambient growth temperature, elevated [CO_2] increased leaf fresh weight by 12%, leaf dry weight by 23%, leaf area by 8%, and specific leaf weight by 4 (leaf fresh weight/leaf area) to 13% (leaf dry weight/leaf area). At high growth temperature, the enhancements in leaf biomass and specific leaf weight

Table 4
Biomass, area and specific weight of fully-developed leaves of 'Florunner' peanut plants grown under 360 and 720 μmol CO_2 mol^{-1} and at average temperatures of 1.5 and 6.0 °C above outdoor ambient temperature (T_A)

$[CO_2]$ ($\mu mol\,mol^{-1}$)	Temperature (°C)	Fresh weight (mg per leaflet)	Dry weight (mg per leaflet)	Area (cm^2 per leaflet)	Fresh weight/area ($mg\,cm^{-2}$)	Dry weight/area ($mg\,cm^{-2}$)
360	T_A + 1.5	262.5 (10.5) b	63.1 (3.9) b	10.2 (0.4) ab	25.7 (1.0) b	6.2 (0.3) bc
	T_A + 6.0	217.5 (15.2) c	49.4 (2.7) c	9.0 (0.3) b	24.3 (1.2) b	5.5 (0.3) c
720	T_A + 1.5	293.1 (12.5) a	77.5 (4.0) a	11.0 (0.6) a	26.6 (1.1) b	7.0 (0.3) b
	T_A + 6.0	296.3 (14.0) a	81.9 (3.8) a	9.9 (0.4) ab	29.8 (1.2) a	8.2 (0.4) a

Measurements were performed 60 days after seed planting. Values are the mean and S.E. (parentheses) of 36 leaflets. Values with different letters in the same column are significantly different at $P < 0.05$ using a Duncan Multiple Range Test.

by elevated $[CO_2]$ were even greater: 36% for fresh weight, 66% for dry weight, 10% for area, and 23 (fresh weight/area) to 49% (dry weight/area) for specific weight.

4. Discussion

This study shows a down-regulation in both activity and protein concentration of Rubisco, expressed on a leaf area basis, in the subtropical peanut plants grown under long-term CO_2 enrichment. Similar declines in Rubisco protein concentration and activity at elevated growth $[CO_2]$ have been reported for other C_3 plant species (Sage et al., 1989; Nie et al., 1995; Van Oosten and Besford, 1995; Drake et al., 1997; Gesch et al., 1998; Moore et al., 1998; Vu et al., 1997, 1999, 2001). In addition to Rubisco, there are also reports that long-term elevated growth $[CO_2]$ affects activities of SPS and ADGP, the control points in sucrose and starch synthesis, respectively (Moore et al., 1998; Hussain et al., 1999; Vu et al., 2001). However, responses of the catalytic proteins to elevated growth $[CO_2]$ are primarily species-specific. Furthermore, there are also variations in acclimation reports for the same species, indicating that plant growth conditions and developmental stages could modify this phenomenon (Bowes, 1993).

In peanut, it has been recently reported that a doubling of ambient $[CO_2]$ enhances leaf photosynthesis by 27% and seed yield by 30% across a range of daytime growth temperatures from 32 to 44 °C (Prasad et al., 2003). There are no effects of elevated $[CO_2]$ on phenology, i.e., days to first flower, pod, seed or maturity. However, these events are sensitive to high temperature. Although the time to first flower is shorter by 3 days at 40 °C compared with 32 °C, the start of pod and seed filling is delayed by 10 days for both ambient and elevated $[CO_2]$ plants grown at 40 °C (Prasad et al., 2003). Craufurd et al. (2002) also reported that the start of pod and seed filling is delayed by 5–9 days when peanut plants are exposed to high daytime temperature (38 °C) from start of flowering to maturity. Similarly, the start of seed filling is progressively delayed by as much as 7 days even when peanut plants are exposed to a short period (6 days) of high daytime temperature between 30 and 45 °C during flowering (Wheeler et al., 1997).

Rubisco protein concentration and activity have been used as biochemical indicators to characterize leaf photosynthetic capacity at elevated growth $[CO_2]$ (Vu et al., 1997; Moore et al., 1999). From the modeled response of leaf CER to changes in CO_2 partial pressure at the chloroplastic site of Rubisco carboxylation (C_c) described by Farquhar and Sharkey (1982), the relationship between CER and C_c is linear and proportional to the in vivo Rubisco protein content/activity at low levels of C_c, which equivalently range from about 100 to 330 $\mu mol\,mol^{-1}$ ambient $[CO_2]$. The initial slope of this CER/C_c relationship is an indicator of the Rubisco carboxylation efficiency. For CO_2-enriched peanut plants, there was reduction in the initial slope of the CER/C_{ca} responses (Fig. 1), and this reduction would reflect decreases in activity as well as protein concentration of Rubisco (Table 2). In addition, there was decline in Rubisco activation for peanut at elevated growth CO_2. These acclimation responses, including a "coarse" control through lowering of the Rubisco protein content and activity and a "fine" control through decreasing activation state and carboxylation efficiency of the enzyme, have been also reported for a variety of C_3 species

grown under a CO_2 enrichment regime (Sage, 1994; Woodrow, 1994; Drake et al., 1997; Vu et al., 1997; Moore et al., 1999).

Despite a decrease in the carboxylation efficiency, photosynthetic capacity and activation of Rubisco, leaf CER and Rubisco photosynthetic efficiency of peanut plants grown at elevated [CO_2] were greater. For C_3 plants, current atmospheric [CO_2] is insufficient to saturate Rubisco activity and larger concentrations of the enzyme appear necessary to support light-saturated photosynthetic rates (Masle et al., 1993). However, as atmospheric [CO_2] is risen to a level that doubles the current concentration, up to 35% of the Rubisco protein could be lost from the leaf before the enzyme would co-limit the photosynthetic rate (Drake et al., 1997). At 700 μmol CO_2 mol^{-1} and a leaf temperature of 25 and 35 °C, only about 59 and 42% of the Rubisco would be required, respectively, to maintain the rate of photosynthesis observed at 350 μmol CO_2 mol^{-1} (Woodrow, 1994). As a protein that can constitute 25% of leaf nitrogen in a C_3 leaf, a decline in the Rubisco protein concentration suggests an optimization of plant nitrogen use, either by reallocating the nitrogen resources away from Rubisco to other proteins within the leaves, or redistributing nitrogen from the photosynthetic proteins of source leaves to sink tissues, under elevated growth [CO_2] (Stitt, 1991; Bowes, 1993; Drake et al., 1997).

Peanut responded, as did many other C_3 crop species, to elevated [CO_2] with an increase in leaf CER and decreases in stomatal conductance (Idso and Kimball, 1992; Bowes, 1993; Rogers and Dahlman, 1993; Drake et al., 1997; Prasad et al., 2003). The reduced stomatal conductance was most likely the result of a direct CO_2 enrichment effect on stomatal aperture, resulting in a reduction in leaf transpiration and consequently an improvement in WUE (Table 1) and tissue water status (Drake et al., 1997; Jarvis et al., 1999). For herbaceous plants, elevated [CO_2] causes an average reduction in stomatal conductance of 36–40% (Field et al., 1995; Norby et al., 1999). In this study, stomatal conductance of fully-expanded mature leaves of peanut plants grown at elevated [CO_2] was about 30 and 44% lower than their ambient-[CO_2] counterparts at near ambient temperature and high temperature, respectively. Gas exchange measurements throughout the growing season for the youngest mature leaves of peanut grown at 28 °C in controlled environment glasshouses also showed that plants of the 700 μmol CO_2 mol^{-1} treatment are 12 and 34% less in transpiration and stomatal conductance, but 77 and 83% higher in CER and WUE, respectively, compared with plants of the 375 μmol CO_2 mol^{-1} treatment (Clifford et al., 2000). With a continued rise in atmospheric [CO_2], leaf CER will be enhanced, at least in the C_3 species, and stomatal conductance will be decreased so plants would use less water and become more efficient in water use (Wullschleger et al., 2002; Hetherington and Woodward, 2003). In peanut, the relatively high stomatal conductance values obtained in this study (Table 1) and by others (Prasad et al., 2003) were likely the result of high leaf photosynthetic rates observed for this plant crop species (Bennett et al., 1993).

In C_3 plants, leaf CER is affected by growth temperature, exerted primarily through Rubisco. High growth temperature reduces, relative to O_2, both solubility of CO_2 and specificity of Rubisco for CO_2, which results in favor of oxygenation and greater losses of CO_2 to photorespiration (Long, 1991). Consequently, a rise in atmospheric [CO_2], and the concomitant inhibition of the Rubisco oxygenase reaction, should alleviate the adverse effects of high temperature on C_3 photosynthesis and result in even greater enhancement of leaf CER by elevated [CO_2] as growth temperature increases (Long, 1991). However, the data in this regard are equivocal (Farrar and Williams, 1991), and the degree of leaf CER enhancement by elevated growth [CO_2] appears to be influenced by other factors, including the temperature optimum for the species, the extent to which Rubisco is down-regulated and the species-specific differences (Vu et al., 1997). This may explain some of the literature reports of species variation in CO_2-enrichment response as a function of temperature. For soybean (*Glycine max* L.), the enhancement effect on leaf CER at double-ambient growth [CO_2] increases linearly from 32 to 95% with increasing day temperatures from 28 to 40 °C, whereas for rice (*Oryza sativa* L.) it stays relatively constant at 60% from 32 to 38 °C (Vu et al., 1997). The present study with peanut showed that the percentage enhancement in leaf CER due to doubling the growth [CO_2] was 35% at $T_A + 1.5$ °C and 14% at $T_A + 6.0$ °C. The percentage enhancement in WUE by elevated [CO_2] was, however, higher: 56% under near ambient and 41% at high temperature (Table 1). At elevated growth

[CO_2], the increase in WUE may be more important than that in CER, especially when soil water content in the root zone area becomes the limiting factor (Chaves and Pereira, 1992).

The results of this study support the hypothesis that, for peanut plants grown at elevated [CO_2], there was a down-regulation of the leaf photosynthetic capacity, manifested through reductions in activity and protein concentration of Rubisco, and an up-regulation of the Rubisco photosynthetic efficiency and carbohydrate metabolism. Because less Rubisco protein is required, the redistribution of nitrogen would increase the efficiency of nitrogen use for peanut under elevated [CO_2]. Besides, the optimization of inorganic carbon acquisition and greater accumulation of the primary photosynthetic products would be beneficial for peanut growth at elevated [CO_2]. Thus, in the absence of other stresses, peanut photosynthesis would perform well under rising atmospheric [CO_2] and temperature predicted for this century. However, one must be cautious in extrapolating high temperature tolerance of leaf photosynthesis and vegetative performance to economic seed yield production, as a recent study shows that decreases in seed yield occur for elevated [CO_2] peanut plants at growth temperatures $\geq 36\,^{\circ}C$ despite greater photosynthesis and biomass accumulation (Prasad et al., 2003).

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